Chapter 4

Variation in a local rice variety, Muey Nawng

4.1 Introduction

In Chapter 2, the local rice variety Muey Nawng was found to be resistant to gall midge and produced higher grain yield than other varieties at Mae Moot village. However, different accessions of Muey Nawng obtained from farmers seed showed varying degrees of susceptibility to tolerance to gall midge in Mae Moot, ranging from no infestation to high infestation in the same order of susceptible check, San-pah-tawng 1 (Chapter 3). In Thailand, the rice gall midge is widespread in the Northern and Northeastern regions. The rice gall midge from different locations was found to differentiate into different biotypes (Katiyar *et al*., 2000). The biotypes of rice gall midge in Thailand have been classified into 3 groups (Thongphak *et al*., 1999; Charapok, 2006). Therefore, the reactions of different rice gall midge biotypes can be very different in different rice varieties (Kalode and Bentur, 1989; Nwilene *et al*., 2002).

Local rice varieties in Northern Thailand are genetically diverse (Rerkasem, 2005). Genetic variations have been found between different accessions bearing the same name and within individual accession as well as between varieties (Meesin, 2003; Supamongkol, 2006; Pintasan *et al*., 2007). It would therefore be useful to examine variation in Muey Nawng accessions. This can be done with molecular

techniques such as RFLP (Zhang *et al*., 1992), AFLP (Paul *et al*., 1997), RAPD (Massawe *et al*., 2003), ISSR (Ghariani *et al*., 2003) and SSR (Chakravarthi and Naravaneni, 2006), with phenotypes and with reactions to gall midge.

Studies in this chapter aimed to evaluate variation in Muey Nawng accessions in 3 ways: (i) examining interaction between the twenty Muey Nawng accessions and six gall midge populations in the level damage; (ii) the twenty Muey Nawng accessions will be examined for variation in morphological and developmental characteristics; and (iii) variation in and between the Muey Nawng accessions will be determined with molecular markers.

4.2 Materials and Methods

4.2.1 Evaluating reaction of twenty Muey Nawng accessions to six populations of gall midge under green house condition

The experiment was carried out at the Entomology and Zoology group, Department Of Agriculture, Bangkok, Thailand, during May 2005 – January 2006 to evaluate damages by six gall midge populations on 20 accessions of Muey Nawng under green house condition (Figure 4.1). Populations of the gall midge collected from six provinces: Tak, Chiang Mai, Chiang Rai, Ubon Ratchathani, Nong Khai and Lumpang were greenhouse-reared and provided by the Entomology and Zoology group (Tayathum *et al*., 1995). Twenty accessions of Muey Nawng (Table 3.1) were grown in cages with check varieties: three resistant (Muey Nawng 62 M, RD4 and RD9) and in two susceptible varieties (RD1 and San-pah-tawng 1). Five day old seedlings of each variety were transplanted into a 10X10X10 cm^3 tray, one tray for one accession or variety. Twenty five trays were placed in a mesh-cover cage

(80X120X90 cm^3), representing one block of a Randomize Completed Block Design. There were four replications. The adult insects were released into the cage 15 days after transplanting, at the rate of 45 insects per cage. Water was sprayed in the cage every two hours from 6 am to 6 pm to maintain high humidity that favors gall midge infestation of the rice plants. Infestation of the gall midge was assessed 30 days later and number of silver shoots determined for the assessment of percentage of gall midge infestation (as in Experiment 2.2.2). Data were analyzed according to the procedure of combined analysis of variance (Gomez and Gomez, 1984). The levels of resistance/susceptibility to rice gall midge refer to the percentage of infestation and are related to the Standard Evaluation System for rice (IRRI, 1996) as given in Table 4.1.

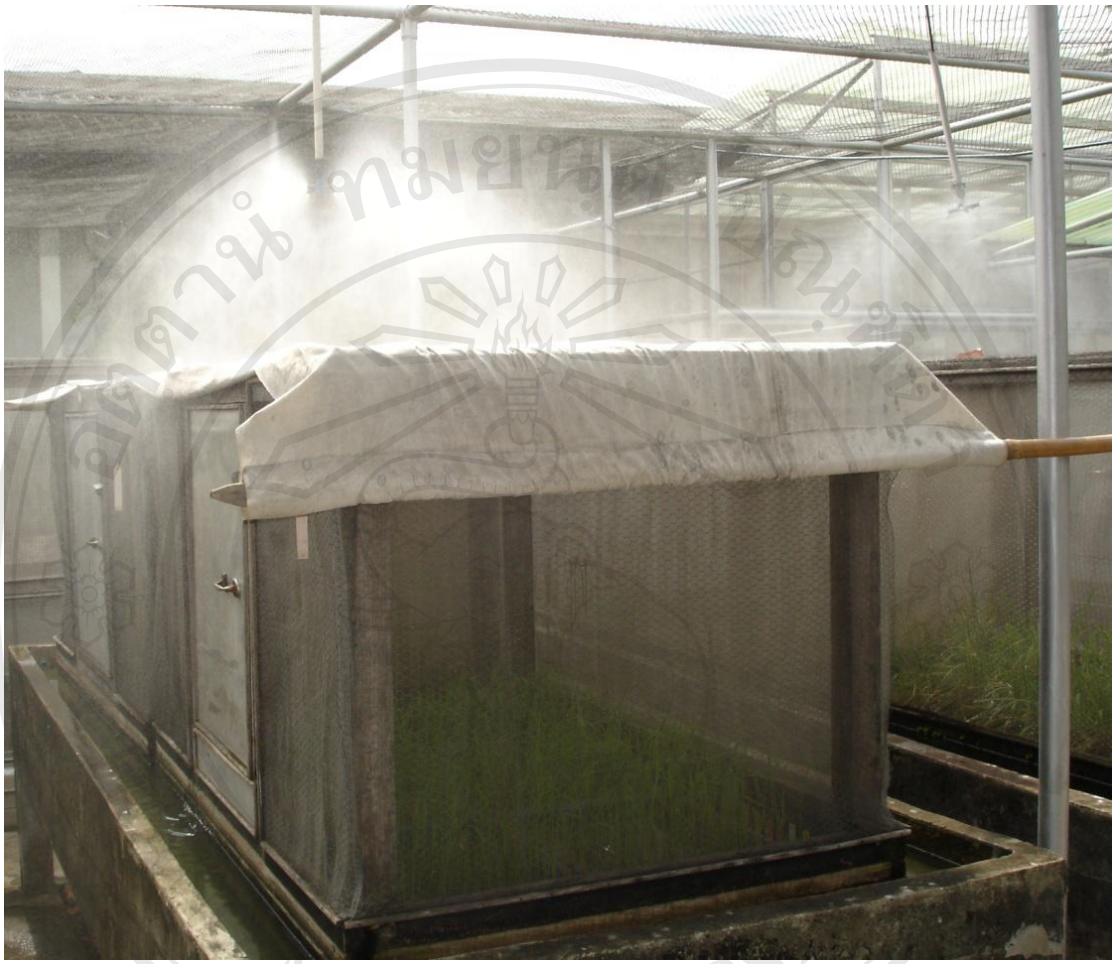

Figure 4.1 The twenty Muey Nawng accessions to gall midge in damage under green house condition.

Table 4.1 The reaction of rice gall midge infestation by the Standard Evaluation System for Rice (IRRI, 1996).

Reactions	**Percent damage**
Highly resistant (HR)	no damage
Resistant (R)	less than 5%
Moderately resistant (MR)	5-10 %
Moderately susceptible (MS)	10-20%
Susceptible (S)	20-50%
Highly susceptible (HS)	more than 50%

4.2.2 Variation of Muey Nawng accessions.

4.2.2.1 Variation in grain quality and morphology

Iodine test

The experiment tested in twenty Muey Nawng accessions collected from farmer's seed same as in Table 3.1. A 120 grains sample of milled rice from each accession were placed in 10.5 cm diameter petri-dish, two grains each of RD4 (glutinous endosperm) and two of KDML105 (non-glutinous endosperm) were placed in each side of petri-dish as checks. Twenty ml of iodine (I) solution (1 g of I_2 KI in 1,000 ml of deionized water) was added to the petri-dish and incubated for 30 minutes at room temperature with the cover on. After that, decanted of liquid and washed by deionized water. A grain appearance and disintegration were visually classified the

glutinous type in brown color grain (negative I reaction) and the non-glutinous type in blue black color grain (positive I reaction (Figure 4.2)).

Alkaline Spreading test

The experiment tested for gelatinization temperature (for softness or hardness of cooked rice) in twenty Muey Nawng accessions collected from farmer's seed same as in Table 3.1. A 120 grain sample of milled rice from each accession were placed in 10.5 cm diameter petridish (20 grains per petri-dish), two of RD4 and two of KDML105 grain were placed in each side of petri-dish as the hard and soft check rice. A 50 ml sample of 1.7% KOH was added to the perti-dish, taking care that the grains did not touch each other. The petri-dishes were covered and incubated for 23 hrs at room temperature. Grain appearance and disintegration were visually rated after incubation, based on the following numeric scale:

Description	**Score**
Grain not affected	1
Grain swollen	2
Grain swollen, collar incomplete or narrow	3
Grain swollen, collar incomplete or wide	4
Grain split or segmented, collar complete and wide	5
Grain dispered, merging with collar	6
Grain completely dispersed and intermingled	7

Check samples with scores of 1-7 were run with each analysis. A rating of 6-7 was classified in this study as soft rice grain, 4-5 classified as medium rice grain, and 2-3 classified as hard rice grain (Figure 4.3).

Figure 4.2 Iodine test stained in brown color glutinous grain and in black color non-glutinous grain of Muey Nawng and two check varieties.

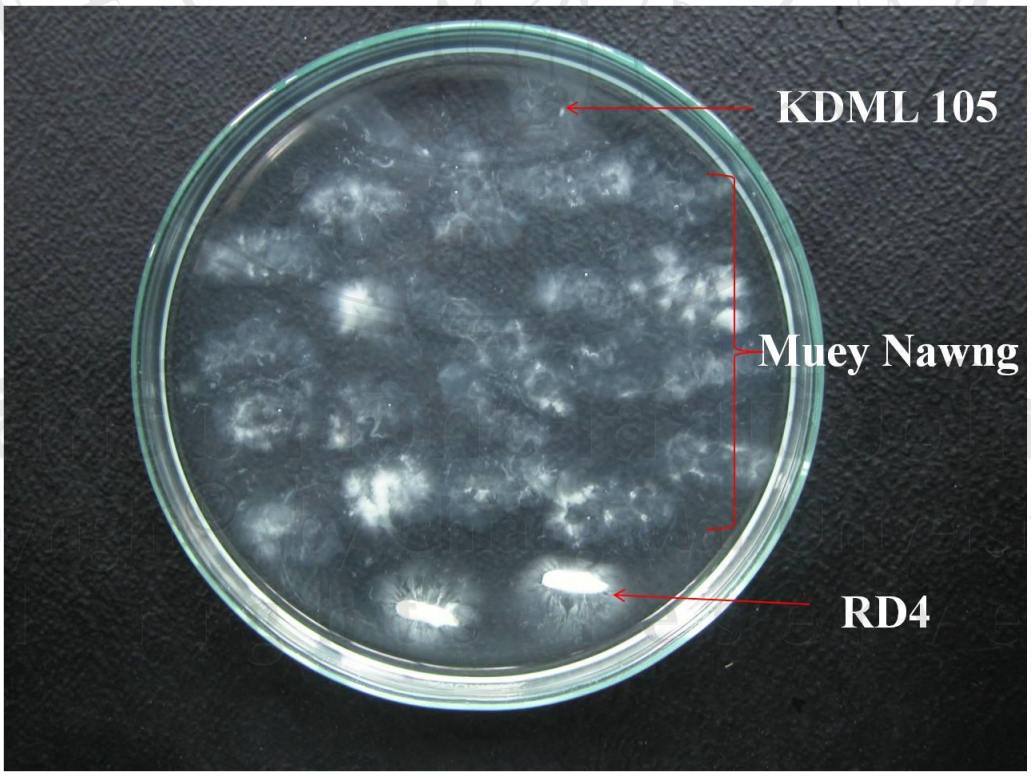

Figure 4.3 Alkaline spreading tested in Muey Nawng and two check varieties.

Morphological characteristics

The seed of 20 Muey Nawng accessions (same as in Table 3.1), one hundred seeds per sample, were characterized in husk color, pericarp color, apiculus color, awning, seed length, seed width and seed thick. After that each accession was germinated in petri-dish before being transplanted 20 plants per accession into pot (10 plants per pot). The transplanting was done on 16st December 2008 until heading date at Agronomy Department, Faculty of Agriculture, Chiang Mai University. The morphological characteristics were recorded individually using the method of IRRI-IBPGR (1980). The plants were recorded at different plant parts including, leaf blade color, leaf sheath color, auricle color, ligule color, ligule shape, internode color, node color, stigma color, plant type and days to heading.

The morphological characters were analyzed by Shannon-Weaver index (*H´)* (Shanon-Weaver, 1949 cited by Power and McSorley 2000) that can be calculated as follow:

$$H' = -\sum_{i=1}^{S} pi \ln pi$$

When *s*= total number of type were found

P_i = proportion of the number of type i divided by total number of plant in each plot

The variation of seed sizes and day of heading were evaluated by standard deviation (SD) and coefficient of variance (CV).

4.2.2.2 Genetic diversity by microsatellite markers

Leaves of twenty Muey Nawng accessions were collected individually, 10 individuals per accession at 45 days after transplanting. Leaves were silica-dried following the method described by Chase and Hill (1991) and stored at -20^{o}C until used. Dried leaves were cut into small pieces and ground into powders in liquid nitrogen. Genomic DNA was isolated individually from the ground leave using modified CTAB method (Doyle and Doyle, 1987).

The 88 microsatellite markers along 12 rice chromosomes were screened in bulk MN6 (highly resistant), and MN9 (highly susceptible). The bulk method was conducted by mixed five randomly selected individual plants DNA per accession. After screening, four polymorphism primer pairs (Table 4.2), RM212, RM21, RM235 and RM309 were chosen for evaluating genetic variation within and between 20 accessions of Muey Nawng. Amplification of DNA were performed in 20 µl reaction consisted of 20 – 50 ng DNA, 0.25 mM of each dNTP, 0.2 µM of each primers and 0.5 unit of Taq DNA polymerase in reaction buffer (10 mM of tris-HCL pH 8.5, 50 mM KCL, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 50% (v/v) glycerol). The amplified products were mixed with loading dye and separated in 10% polyacrylamide gel electrophoresis. Gel was stained with ethidium bromide and photographed under UV transilluminators.

Table 4.2 Chromosomal locations, primer sequences, repeat motif and annealing temperature of four microsatellite primers.

Chormosomal location	Name	Primer sequences (5 → 3)	Repeat Motif	Annealing temperature (°C)	References
1	RM212	F- CCACTTTCAGCTACTACCAGAA R- CATAATGAGAGACAAATGGGTG	$(CT)_{24}$	55	Chen *et al*., 1997
11	RM21	F-ACAGTATTCCGTAGGCACGG R-GCTCCATGAGGGTGGTAGAG	$(GA)_{18}$	55	Panaud *et al*., 1996
12	RM235	F-AGAAGCTAGGGCTAACGAAC R-TCACCTGGTCAGCCTCTTTC	$(CT)_{24}$	55	Chen *et al*., 1997
12	RM309	F-GTAGATCACGCACCTTTCTGG R-AGAAGGCCTCCGGTGAAG	$(GT)_{13}$	55	Temnykh *et al*., 2000

* F- forward primer, R-Reverse primer

The DNA analysis of the genetic diversity index (H_e) was calculated for each rice population according to Nei (1973) as;

$$H_e = 1 - \sum P_i^2$$

Where P_i is the alleles frequency.

The variation of between and within accession was calculated according to Nei *et al*., (1983) for each microsatellite locus. The total diversity estimate (H_T) was partitioned into within population diversity (H_S) and between population diversity (D_{ST}) components, where $H_T = H_S + D_{ST}$. Gene diversity between populations was expressed as relative to total population diversity or genetic differentiation index (F_{ST}), where $F_{ST} = D_{ST} / H_T$, according to Nei and Kumar (2000). Analysis of genetic diversity indices were performed using POPGENE version 3.2.

4.3 Results

4.3.1 Evaluating reaction of twenty accessions of Muey Nawng to six populations of gall midge infestation under green house condition

The interaction between different accessions of Muey Nawng and different populations of gall midge was highly significant, $P < 0.001$ (Table 4.3). When looking at each population of gall midge, the percentage of infestation in twenty Muey Nawng accessions by the gall midge form Tak varied form no infestation to 88.6%. The accession that was completely free of gall midge infestation was MN6. With the gall midge form Nong Khai, the percentage of infestation in twenty Muey Nawng accessions was varied from 2.4% to 89.8%. With the gall midge from Chiang

Rai, the percentage of infestation in twenty Muey Nawng accessions was varied from no infestation to 70.4 %. No infestation was observed in MN3, MN5, MN6, MN11, MN13 and Muey Nawng 62 M. In gall midge from Ubon Ratchathani, the percentage of infestation in twenty Muey Nawng accessions was varied from no infestation to 45.1 %. No infestation by Ubon Rathchathani gall midge was found in MN6 and Muey Nawng 62 M. In gall midge form Chiang Mai, the percentage of gall midge infestation of twenty Muey Nawng accessions was varied from no infestation to 38.2 %. No infestation by Chiang Mai gall midge was found in MN5, MN6. In population of gall midge from Lumpang, the percentage of gall midge infestation of twenty Muey Nawng accessions was varied from no infestation to 34.7 %. No infestation was found in MN6 and MN11. In check varieties, Muey Nawng 62 M was the no infestation to gall midge from Chiang Rai and Ubon Ratchathani but it was few percentage infestation in 1.3%, 1.6%, 7.9% and 9.7% to gall midge form Chiang mai, Lumpang, Tak and Nong Khai, respectively. In addition, the percentage of infestation in RD1, RD4, RD9 and San-pah-tawng1 had nearly or more than 50 % in all gall midge population, except, the percentage of infestation by gall midge from Ubon Ratchathani, Chiang Mai and Lumpang in RD4 had less than 50%.

According to the Standard Evaluation System for Rice (IRRI, 1996), the Muey Nawng accessions varied from highly susceptible up to highly resistant to the six gall midge populations (Table 4.4). For the check varieties, RD1 and San-pah-tawng 1 were highly susceptible to all populations of gall midge, except San-pah-tawng 1 which was susceptible to gall midge from Lumpang. RD4, released as resistant to gall midge was highly susceptible to gall midge from Tak, Nong Khai and Chiang Rai, susceptible gall midge from Ubon Ratchathani and Chiang Mai and moderately

susceptible gall midge form Lumpang. Muey Nawng 62 M was the highly resistant to gall midge form Chiang Rai and Ubon Ratchathani, resistant to gall midge form Nong Khai, Chiang Mai and Lumpang and moderately resistant to gall midge form Tak. On the other hand, average infestation across the accessions was highest with Tak gall midge, followed by Nong Khai, Chiang Rai, Ubon Ratchathani, Chiang Mai and Lumpang, in descending order.

Table 4.3 Gall midge damage (%) in 20 Muey Nawng accessions and five check varieties by six populations of gall midge under green house condition.

Variety	Gall midge population						
	Tak	Nong Khai	Chiang Rai	Ubon Ratchathani	Chiang Mai	Lumpang	Mean
Accessions							
MN1	21.7	34.9	24.3	15.4	13.5	18.7	21.2
MN2	26.0	13.2	18.1	13.5	13.7	7.8	15.0
MN3	10.1	14.7	0	5.7	2.9	1.2	5.5
MN4	35.0	19.2	18.8	9.7	7.6	4.0	14.7
MN5	14.9	8.3	0	2.4	0	1.3	3.4
MN6	0	3.6	0	0	0	0	0.5
MN7	19.4	26.7	15.0	35.3	16.1	5.5	19.4
MN8	77.5	71.2	68.9	41.5	23.8	37.8	51.8
MN9	88.6	89.8	70.4	41.0	32.6	29.3	58.3
MN10	36.8	15.0	26.8	14.7	14.3	15.7	20.3
MN11	17.0	2.4	0	2.5	2.8	0	4.0
MN12	32.3	25.8	28.6	18.6	5.6	14.3	19.2
MN13	18.8	4.6	1.3	7.6	4.4	1.1	6.0
MN14	13.4	30.7	21.4	12.9	7.7	6.6	13.7
MN15	57.6	65.4	41.7	39.2	38.2	17.7	42.0
MN16	22.8	24.8	23.9	7.2	13.1	13.1	16.7
MN17	32.0	10.1	15.9	13.6	6.3	6.3	13.6
MN18	15.8	34.2	17.7	14.4	10.3	3.8	14.1
MN19	73.7	70.8	27.1	45.1	16.5	34.7	41.6
MN20	34.9	12.6	21.6	26.1	6.3	8.9	17.8

Variety	Gall midge population						
	Tak	Nong Khai	Chiang Rai	Ubon Ratchathani	Chiang Mai	Lumpang	Mean
Check varieties							
Muey Nawng							
62 M	7.9	9.7	0	0	1.3 I	1.6	2.1
RD1	89.6	97.4	91.3	81.8	81.9 A	69.8	85.2
RD4	70.2	76.9	54.2	34.1	24.9 BC	12.6	45.2
RD9	84.4	92.8	73.5	84.5	84.9 A	48.2	77.7
San-pah-tawng 1	89.3	92.6	83.4	75.2	62.5 A	48.6	74.9
Mean	35.1	28.6	25.5	21.0	14.9	13.6	
	Rice accession/variety (V)			Gall midge population (G)			V X G
F-test	*			*			*

*=significant at P<0.05. V x G indicated F-test for rice accession/variety and gall midge population interaction. The difference between varieties in same column is indicated by lower case letters.

Table 4.4 The reactions of 20 Muey Nawng accessions and five check varieties to six populations of gall midge under greenhouse condition were classified by Standard Evaluation System for Rice (IRRI, 1996).

Variety	**Gall midge population**					
	Tak	Nong Khai	Chiang Rai	Ubon Ratchatahni	Chiang Mai	Lumpang
Accessions						
MN1	S	S	S	MS	MS	MS
MN2	S	MS	MS	MS	MS	MR
MN3	MR	MS	HR	MR	R	R
MN4	S	MS	MS	MR	MR	R
MN5	MS	MR	HR	R	HR	R
MN6	HR	R	HR	HR	HR	HR
MN7	MS	S	MS	S	MS	MR
MN8	HS	HS	HS	S	S	S
MN9	HS	HS	HS	S	S	S
MN10	S	S	S	MS	MS	MS
MN11	MS	R	HR	R	R	HR
MN12	S	MS	S	MS	MR	MS
MN13	MS	R	R	MR	R	R
MN14	MS	MS	S	MS	MR	MR
MN15	HS	HS	S	S	S	MS
MN16	S	S	S	MR	MS	MS
MN17	S	MR	MS	MS	MR	MR
MN18	MS	S	MS	MS	MS	R
MN19	HS	HS	S	S	MS	S
MN20	S	MR	S	S	MR	MR

Variety	Gall midge population					
	Tak	Nong Khai	Chiang Rai	Ubon Ratchatahni	Chiang Mai	Lumpang
Check varieties						
Muey Nawng 62 M	MR	R	HR	HR	R	R
RD1	HS	HS	HS	HS	HS	HS
RD4	HS	HS	HS	S	S	MS
RD9	HS	HS	HS	HS	HS	S
San-pah-tawng 1	HS	HS	HS	HS	HS	S

Note: HR (highly resistant: no silver shoot), R (resistant: 0-5 % damage), MR (moderately resistant: 5-10 % damage), MR (moderately susceptible: 10-20 % damage), S (susceptible: 20-50 % damage) and HS (highly susceptible: more than 50 % damage).

4.3.2 Variation of Muey Nawng accessions

4.3.2.1 Variation in grain quality and morphology

Grain quality

The results of iodine test showed variation of non-glutinous and glutinous grain in twenty Muey Nawng accessions (Table 4.5). Muey Nawng is a glutinous rice variety, however the presence of non-glutinous grain was found in 16 of the 20 accessions, ranging from 0.8 – 55.8 %. MN7, MN10, MN13 and MN16 were the four accessions that showed 100% negative reaction to Iodine, as were the check varieties, Muey Nawng 62M, San-pah-tawang 1 and RD4. The highest percentage of non-glutinous grain was in MN15 (55.8 %) followed by MN19 (45.0%), MN1 (25 %), MN2 (25%), MN12 (25%) and MN8 (7.5), respectively. The other accessions had fewer than 2 % non-glutinous grain.

The alkaline spreading value of 20 Muey Nanwg accessions ranged form 5-7 (Table 4.5). Six accessions, MN1, MN2, MN7, MN12, MN15 and MN19 rated 5-7 of alkaline spreading value. The other accessions were rated 6-7 of alkaline spreading value, indicating that they were mostly soft textured rice when cooked. In check varieties, Muey Nawng 62 M and San-pah-tawng 1 rated 6-7, but RD4, which is considered hard textured rice was rated 1-2 in alkaline spreading value.

Seed characters

There was no variation in pericarp color (white color) and awning (no awning) in both within and between 20 Muey Nawng accessions (data not showed). But the genetic differentiation was found in husk color and apiculus color (Table 4.6). The genetic variation of Shannon-Weaver Index (*H´*) in husk color (straw and straw with

brown line) of 20 Muey Nawng accession was ranged from 0.03 in MN20 (lowest) and 0.53 in MN8 (highest). In addition, the genetic variation of Shannon-Weaver Index in apiculus color (white and purple) of 20 Muey Nawng accessions was ranged from 0-0.68. MN3, MN10, MN16 and MN17 were purple color of apiculus ($H' = 0$) and MN15 and MN19 was the highest. In check varieties, the husk color was the straw color in Muey Nawng 62 M and San-pah-tawng1 and but it was the reddish brown color in RD4. The apiculus color was the purple color in Muey Nawng 62M and RD4 but it was the white color in San-pah-tawng 1. In addition, the husk and apiculus color was not showed the genetic variation within rice varieties.

Morphological characters

All of 20 Muey Nawng accessions and Muey Nawng 62 M showed colorless auricle, 2 – cleft ligule shape, erect plant type, green node and internode (data not showed). But four characters including color of leaf blade, leaf sheath, ligule and stigma were found to vary within accessions of only four Muey Nawng accessions (Table 4.7). The Shannon-Weaver Index (H') in color of leaf blade (green, green with purple at margin), leaf sheath (green, green with purple line), ligule (white, green with purple at margin) and stigma (white, purple) were highest in MN4 (0.69) and MN14 (0.69), intermediate in MN18 (0.67) and lowest in MN7 (0.5). The other accessions and Muey Nawng 62 M showed green with purple at margin leaf blade, green with purple line leaf sheath, green with purple at margin and purple stigma. When planted on 16th December 2008, the average time to overall days of heading in the 20 Muey Nawng accessions ranged from 78.9 days (MN1) to 85.2 days (MN8), compared with

79.7 days in MN 62M. Considerable variation was found within accessions, with coefficient of variation ranging from 0.45% in MN14 to 4.18% in MN9 (Table 4.8).

Seed shape: length, width and thickness

Average grain width in MN1 (3.73 mm) was the lowest and the highest in MN17 (3.95 mm). Coefficient of variation within accessions varied from 3.68% (MN17) to 3.92% (MN 20) (Table 4.9).

Average grain length was the lowest in MN1 (8.95 mm) and the highest in MN10 (9.68 mm). Coefficient of variation within accessions varied from 3.74% (MN11) to 9.68% (MN 10) (Table 4.9).

Average grain thickness was the lowest in MN2 (2.16 mm) and the highest in MN19 (2.32 mm). Coefficient of variation of each accessions varied from 4.70 10.29% (MN20) (Table 4.9).

Table 4.5 The percentage of non-glutinous, glutinous and alkaline value in 20 Muey Nawng accession and three check varieties.

Accessions	Non-glutinous (%)	Glutinous (%)	Alkaline value
MN1	25.0	75.0	5-7
MN2	25.0	75.0	5-7
MN3	1.7	98.3	6-7
MN4	0.8	99.2	6-7
MN5	1.7	98.3	6-7
MN6	1.7	98.3	6-7
MN7	0	100	6-7
MN8	7.5	92.5	5-7
MN9	1.7	98.3	6-7
MN10	0	100	6-7
MN11	1.7	98.3	6-7
MN12	25.0	75.0	5-7
MN13	0	100	6-7
MN14	0.8	99.2	6-7
MN15	55.8	44.2	5-7
MN16	0	100	6-7
MN17	0.8	99.2	6-7
MN18	1.7	98.3	6-7
MN19	45.0	55.0	5-7
MN20	0.8	99.2	6-7
Check varieties			
Muey Nawng 62 M	0	100	6-7
San-pah-tawng 1	0	100	6-7
RD4	0	100	1-2

Alkaline spreading value: a rating of 6-7 was classified as soft rice grain, 4-5 classified as medium rice grain and 1-3 classified as hard rice grain.

Table 4.6 Seed characters and Shannon's Index (*H´*) of 20 Muey Nawng accessions and three check varieties.

	Husk color	*H´*	Apiculus color	*H´*
Accessions				
MN1	straw, straw with brown line	0.38	white, purple	0.56
MN2	straw, straw with brown line	0.24	white, purple	0.57
MN3	straw, straw with brown line	0.51	purple	0
MN4	straw, straw with brown line	0.31	white, purple	0.03
MN5	straw, straw with brown line	0.49	white, purple	0.03
MN6	straw, straw with brown line	0.21	white, purple	0.04
MN7	straw, straw with brown line	0.20	white, purple	0.08
MN8	straw, straw with brown line	0.53	white, purple	0.31
MN9	straw, straw with brown line	0.31	white, purple	0.06
MN10	straw, straw with brown line	0.12	purple	0
MN11	straw, straw with brown line	0.15	white, purple	0.06
MN12	straw, straw with brown line	0.08	white, purple	0.59
MN13	straw, straw with brown line	0.18	white, purple	0.03
MN14	straw, straw with brown line	0.30	white, purple	0.06
MN15	straw, straw with brown line	0.12	white, purple	0.68
MN16	straw, straw with brown line	0.18	purple	0
MN17	straw, straw with brown line	0.42	purple	0
MN18	straw, straw with brown line	0.12	white, purple	0.03
MN19	straw, straw with brown line	0.23	white, purple	0.68
MN20	straw, straw with brown line	0.03	white, purple	0.03
Check varieties				
Muey Nawng 62 M	straw	0	purple	0
San-pah-tawng 1	straw	0	white	0
RD4	reddish brown	0	purple	0

Table 4.7 Morphological characters and Shannon's Index (*H´*) of 20 Muey Nawng accessions and check variety.

	Leaf blade color	***H´***	**Leaf sheath color**	***H´***	**Ligule color**	***H´***	**Stigma color**	***H´***
Accession								
MN1	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN2	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN3	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN4	green, green with purple at margin	0.69	green, green with purple line	0.69	write, write with purple at margin	0.69	white, purple	0.69
MN5	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN6	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN7	green, green with purple at margin	0.50	green, green with purple line	0.50	write, write with purple at margin	0.50	white, purple	0.50
MN8	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN9	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN10	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN11	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN12	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN13	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN14	green, green with purple at margin	0.69	green, green with purple line	0.69	write, write with purple at margin	0.69	white, purple	0.69
MN15	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0

	Leaf blade color	*H´*	Leaf sheath color	*H´*	Ligule color	*H´*	Stigma color	*H´*
MN16	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN17	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN18	green, green with purple at margin	0.67	green, green with purple line	0.67	write, write with purple at margin	0.67	white, purple	0.67
MN19	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
MN20	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0
Check variety								
Muey Nawng 62 M	green with purple at margin	0	green with purple line	0	write with purple at margin	0	purple	0

Table 4.8 Days to heading in 20 Muey Nawng accessions and check varieties planted at Chiang Mai on December 16th, 2009.

	Days to heading			
	Range	Mean	SD	CV (%)
Accessions				
MN1	77 - 80	78.8	0.8	1.10
MN2	78 - 84	80.1	2.0	2.50
MN3	78 - 81	79.5	1.1	1.38
MN4	79 - 87	82.6	3.3	4.02
MN5	78 - 82	80.2	1.3	1.74
MN6	78 - 82	80.9	1.3	1.67
MN7	79 - 87	82.4	1.9	2.40
MN8	81 - 87	85.2	1.9	2.31
MN9	76 - 87	83.4	3.4	4.17
MN10	77 - 82	81.6	2.1	2.64
MN11	79 - 82	80.5	0.9	1.23
MN12	78 - 82	80.0	1.1	1.48
MN13	78 - 81	80.6	1.4	1.76
MN14	81 - 82	81.8	0.3	0.44
MN15	81 - 87	82.3	1.3	1.63
MN16	81 - 85	82.8	1.1	1.33
MN17	82 - 84	82.4	0.6	0.82
MN18	81 - 86	83.6	1.4	1.70
MN19	81 - 87	84.0	2.1	2.54
MN20	82 - 85	83.9	1.5	1.79
Check variety				
Muey Nawng 62 M	78 - 80	79.7	0.6	0.84

Table 4.9 Grain width, grain length and grain thick between and within of 20 Muey Nawng accessions and three check varieties.

	Grain width (mm)*				Grain length (mm)*				Grain thick (mm)*			
	Mean	**Range**	**SD**	**CV%**	**Mean**	**Range**	**SD**	**CV%**	**Mean**	**Range**	**SD**	**CV%**
Accessions												
MN1	3.73	2.59-4.34	0.22	5.92	8.95	7.41-10.33	0.57	6.35	2.18	1.58-2.58	0.16	7.41
MN2	3.89	3.37-4.44	0.21	5.37	9.29	8.29-10.25	0.39	4.19	2.16	1.32-2.51	0.17	8.07
MN3	3.94	3.27-4.24	0.16	4.17	9.38	8.40-10.36	0.43	4.54	2.25	1.51-2.54	0.16	7.16
MN4	3.88	2.49-4.24	0.23	6.06	9.24	8.29-10.35	0.42	4.59	2.20	1.78-2.53	0.14	6.59
MN5	3.92	3.04-4.22	0.16	3.97	9.31	8.38-10.38	0.37	3.96	2.23	1.59-2.48	0.13	5.97
MN6	3.88	3.25-4.42	0.18	4.56	9.28	8.27-10.29	0.41	4.44	2.20	1.51-2.43	0.16	7.45
MN7	3.84	3.02-4.26	0.24	6.28	9.27	7.72-10.35	0.50	5.34	2.25	1.66-2.54	0.13	5.90
MN8	3.76	3.09-4.13	0.17	4.64	9.19	8.09-10.22	0.41	4.44	2.22	1.77-2.46	0.12	5.46
MN9	3.81	3.09-4.22	0.16	4.30	9.01	7.90-10.09	0.40	4.43	2.18	2.54-1.73	0.12	5.59
MN10	3.94	3.54-4.89	0.15	3.70	9.68	8.40-11.52	0.69	7.13	2.24	1.86-2.60	0.13	5.71
MN11	3.94	2.68-4.32	0.19	4.71	9.30	8.43-10.33	0.35	3.74	2.26	1.68-2.70	0.13	5.62
MN12	3.87	3.25-4.19	0.15	3.80	9.29	7.50-10.33	0.42	4.55	2.24	1.81-2.46	0.12	5.57
MN13	3.93	3.10-4.37	0.17	4.36	9.28	8.44-10.02	0.35	3.79	2.22	1.68-2.49	0.12	5.32
MN14	3.89	3.15-4.39	0.18	4.58	9.34	8.30-10.30	0.39	4.15	2.25	1.95-2.54	0.11	4.70
MN15	3.76	2.62-4.26	0.21	5.65	9.05	8.16-10.11	0.41	4.53	2.19	1.77-2.48	0.13	5.83
MN16	3.90	3.19-4.24	0.17	4.47	9.34	8.45-10.16	0.37	3.95	2.22	1.60-2.48	0.12	5.34
MN17	3.95	3.49-4.32	0.15	3.68	9.28	8.37-10.70	0.43	4.59	2.25	1.78-2.53	0.12	5.50
MN18	3.93	3.08-4.50	0.19	4.95	9.56	8.48-10.92	0.45	4.70	2.24	1.76-2.57	0.14	6.29
MN19	3.92	3.23-4.88	0.21	5.45	9.33	8.13-10.31	0.41	4.41	2.32	1.79-2.62	0.13	5.56
MN20	3.90	3.08-4.35	0.27	6.92	9.40	8.24-10.55	0.48	5.07	2.21	1.32-2.57	0.23	10.29

4.3.2.2 Genetic variation detected by microsatellite markers

Twenty Muey Nawng accessions illustrated various level of allele variation within and among accessions (Table 4.10). The highest number of alleles were detected in RM21 (5 alleles), following with RM235 (4 alleles), and the lowest in RM309 and RM212 (3 alleles). At each locus, RM212 and RM21 detected genetic variation in six accessions, while RM235 and RM309 detected genetic variation in ten accessions.

Gene diversity index (H_e) was observed at each locus (Table 4.11). Genetic variation was found in 13 accessions ranging from 0.117 in MN14 and MN17 to 0.489 in MN19 was the highest while the others seven accessions showed no variation at these four microsatellite loci (H_e=0). For each locus, the highest mean genetic diversity was illustrated in RM309 followed by RM235, RM21 and RM212 at 0.22, 0.20, 0.15 and 0.11, respectively. The gene diversity index of RM21 was observed in seven accessions ranging from 0.32 to 0.50, MN9 was the lowest and MN15 was the highest but no variation was detected in the rest 13 accessions. In RM309, the gene diversity index was observed in 10 accessions ranging from 0.32 to 0.5, MN1, MN4 and MN8 were the lowest and MN19 was the highest but no variation was detected in the rest 10 accessions. In RM212, gene diversity index within accession was detected in six accessions ranging from 0.18 to 0.48, MN4 was the lowest and MN19 was the highest but no variation was detected in the rest 14 accessions. In RM235, the gene diversity index was detected in 10 accessions ranging from 0.18 to 0.48, MN1 was the lowest and MN2, MN7, MN10, MN16, MN17 and MN20 were the highest but no variation was detected in the rest 10 accessions.

In conclusion, moderated to high level of total genetic variation and average gene diversity within accession was found in 20 local Muey Nawng accessions (H_T =0.286 and H_S=0.173) while gene diversity between accessions (D_{ST}) was 0.108. In addition, the genetic differentiation among 20 MN accessions (F_{ST}) was 0.40. It is showed that approximately 60% of the genetic variation of Muey Nawng was found within individual accessions, genetic differentiation between accessions accounted for less than half.

Table 4.10 Allele frequencies of 20 Muey Nawng accessions at four microsatellite loci.

Accessions	Locus														
	RM212			RM21					RM235				RM309		
	Allele A	Allele B	Allele C	Allele A	Allele B	Allele C	Allele D	Allele E	Allele A	Allele B	Allele C	Allele D	Allele A	Allele B	Allele C
MN1	0.8	0.2	0	0.6	0.2	0.2	0	0	0.9	0.1	0	0	0.8	0.2	0
MN2	1	0	0	1	0	0	0	0	0.7	0.3	0	0	0.7	0.3	0
MN3	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
MN4	0.9	0	0.1	1	0	0	0	0	1	0	0	0	0.8	0.2	0
MN5	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
MN6	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
MN7	0.7	0	0.3	1	0	0	0	0	0.7	0	0	0.3	0.7	0.3	0
MN8	0.8	0.2	0	0.6	0	0.4	0	0	0.8	0	0	0.2	0.2	0.8	0
MN9	0.2	0.8	0	0.2	0	0.8	0	0	0.2	0	0	0.8	0	1	0
MN10	1	0	0	0.7	0	0.3	0	0	0.7	0	0	0.3	0.7	0	0.3
MN11	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
MN12	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
MN13	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
MN14	1	0	0	1	0	0	0	0	1	0	0	0	0.7	0.3	0
MN15	1	0	0	0.5	0.5	0	0	0	1	0	0	0	1	0	0
MN16	1	0	0	1	0	0	0	0	0.7	0	0.3	0	0.7	0.3	0
MN17	1	0	0	1	0	0	0	0	0.7	0	0	0.3	1	0	0
MN18	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
MN19	0.4	0.6	0	0.7	0	0	0.2	0.1	0.2	0.8	0	0	0.5	0.5	0
MN20	1	0	0	1	0	0	0	0	0.7	0.3	0	0	0.7	0.3	0

Table 4.11 Genetic diversity of 20 Muey Nawng (MN) accessions at four microsatellite loci.

Accessions	Gene diversity index (H_e)				Total H_e	H_S	H_T	D_{ST}	F_{ST}
	RM 21	RM 309	RM 212	RM 235					
MN1	0.560	0.320	0.320	0.180	0.384				
MN2	0	0.420	0	0.420	0.234				
MN3	0	0	0	0	0				
MN4	0	0.320	0.180	0	0.139				
MN5	0	0	0	0	0				
MN6	0	0	0	0	0				
MN7	0	0.420	0.420	0.420	0.350				
MN8	0.480	0.320	0.320	0.320	0.400				
MN9	0.320	0	0.320	0.320	0.267				
MN10	0.420	0.420	0	0.420	0.350				
MN11	0	0	0	0	0				
MN12	0	0	0	0	0				
MN13	0	0	0	0	0.000				
MN14	0	0.420	0	0	0.117				
MN15	0.500	0	0	0	0.139				
MN16	0	0.420	0	0.420	0.234				
MN17	0	0	0	0.42	0.117				
MN18	0	0	0	0	0				
MN19	0.460	0.500	0.480	0.320	0.489				
MN20	0	0.420	0	0.420	0.234				
Total MN	**0.152**	**0.221**	**0.113**	**0.204**	**0.173**	**0.173**	**0.286**	**0.108**	**0.398**

H_S = genetic diversity within accession, D_{ST} = gene diversity between accession, H_T = total gene diversity, F_{ST} = genetic differentiation among accession.

4.4 Discussion

Rice varieties vary in the reaction of infestation by different gall midge biotypes (Kalode and Bentur, 1989; Nwilene *et al*, 2002). In this study I have found variation in response to gall midge in twenty accessions of one local rice variety, Muey Nawng. The results confirm in Chapter 3 showed that the variation of 20 Muey Nawng accessions was the no infestation to high infestation by rice gall midge at Mae Moot village. Other have reported that gall midge from different locations in Thailand had different effects on different rice varieties (Tayathum *et al*., 1995). These authors reported that three populations of gall midge collected from Nan, Ubon Ratchathani and Chachengsao provinces in Thailand had variation of percentage of gall midge infestation and levels of resistance/susceptibility different virulence on nine rice varieties. Similarly, the interaction between gall midge populations and rice varieties was correlated the gene-for-gene hypothesis such as the studies of the ten-allelic gall midge resistance genes and six different biotypes of gall midge in India (Sardesai *et al*., 2001). Most importantly, in this study I have found a very strong ($P < 0.001$) interaction between the 20 accessions of Muey Nawng and the 6 gall midge populations. Different gall midge populations had different effects on the different Muey Nawng accessions. For example MN16 and MN20 were found to be moderately resistance (7.2% infestation) and susceptible (26.1% infestation) to gall midge from Ubon Ratchathani but were found susceptible (24.8% infestation) and moderately resistance (12.6% infestation) to gall midge from Nong Khai.

The genetic variation of local rice varieties have been found in morphological character of within accessions (Meesin, 2003; Supamongkol, 2006; Pintasan *et al*., 2007). The genetic variation of within accession was difficult to separate off among

accessions of local rice varieties by visual observation. This study, the genetic variation of Muey Nawng accessions was found in two characters of seed (husk and apiculus color) and in four characters of morphological (color of leaf blade, leaf sheath, ligule and stigma) and days to heading). Moreover, the variation of grain quality within accession was found in percentage of glutinous and non-glutinous grains. However, the genetic variation was clearly detected by DNA analysis. The genetic diversity index (H_e) was found in 14 accessions only using four microsatellite loci. Indeed, gene diversity within accession (H_S) was 0.17 and between accessions was 0.11. In addition, the genetic differentiation among accessions of Muey Nawng (F_{ST}) was 0.40. The genetic different of local Muey Nawng accessions has been found by many causes. The seed of different local rice varieties kept and perpetuated by farmers in many rice growing areas is indicated by rice gall midge in specific of locations. In addition, over time of farmers are modifying the genetic variation of accession by selecting for plants with preferred agro-morphological characteristics. Therefore, this work clearly information only the genetic variation of local varieties in DNA level, DNA markers that associated with gall midge resistance in rice varieties should be need more study.

In conclusions, the different Muey Nawng accessions responded differently gall midge populations from six different locations. One of these, MN 6, was resistant to all six gall midge populations. Gall midge from Tak was most virulent among the population tested. The variation of 20 Muey Nawng accessions may be found between different accessions recognized as a same name and within individual accessions.